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13. ABSTRACT (Maximum 200 words)  A genomics approach yielded a new skeletogenic gene of the sea urchin embryo. This gene, SM37, is expressed coordinately with a gene encoding a known skeletogenic protein (SM50 gene) isolated earlier in our laboratory. Like SM50, SM37 is expressed only in skeletogenic cells. It encodes a protein that includes many copies of a glycine-rich repeat motif, similar in organization but different in sequence from that encoded by the SM50 gene. SM37 and SM50 are linked in the genome at a distance of about 12 kb, and each gene processes its own cis-regulatory system. These systems include the same sequence elements, and apparently function the same way during skeletogenesis.				
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## FINAL REPORT

Grant #: N00014-93-1-0379

PRINCIPAL INVESTIGATOR: Eric H. Davidson

INSTITUTION: California Institute of Technology

GRANT TITLE: The Molecular Biology of the Sea Urchin Skeletal Matrix

AWARD PERIOD: 2/1/96 - 1/31/99

OBJECTIVE: To clone previously unknown genes encoding skeletogenic proteins of *Strongylocentrotus purpuratus* spicular biomineral.

APPROACH: Skeletogenic genes were sought by three different means: *i*, Isolation of matrix proteins from 2D gels and cloning by direct microsequencing (biochemical approach); *ii*, Use of a new technology for "printing" DNA inserts of arrayed cDNA libraries which can be screened directly with skeletogenic mesenchyme cDNA (differential array screening); and *iii*, Isolation of genomic fragments that contain identified target sites for skeletogenic transcriptional regulators, and recovery of associated genes (regulatory target site screening).

ACCOMPLISHMENTS: The three approaches to isolation of skeletogenic genes have been thoroughly explored during the three year tenure of this ONR grant. Conclusions as to their usefulness are as follows: *(i) Biochemical approach:* This approach turns out to be the most difficult to apply to isolation of skeletogenic proteins, because of the nature of these proteins. First, the methods are of high sensitivity and thus even minor contaminants can be recovered. Thus it is necessary to utilize very clean preparations of spicules free of cellular contaminants, or of adult spines. The latter have low protein content, however. Second, because of the internally repetitive nature of many skeletogenic proteins a given protein may generate many proteolytic fragments that contain the same sequence, greatly complicating analysis. Third, the proteins are largely insoluble and also have a great tendency to aggregate. They require complex treatments before reproducible separation by HPLC is feasible. Though we eventually developed methods for accomplishing such separations, it became apparent that molecular approaches are more rapid and powerful. *(ii) Differential array screening for skeletogenic transcripts:* This is by far the most powerful approach to isolation of skeletogenic genes. During the period of this grant we developed technology for differential high C<sub>0</sub>t screening of 22x22 cm robotically arrayed cDNA clone libraries. Though ONR support terminated before this approach could be brought to fruition, in collaboration with Prof. F. Wilt of UC Berkeley we now have generated probes representing those skeletogenic mesenchyme mRNA sequences expressed only on addition to cultured mesenchyme cells of serum (serum factors are needed for skeletogenesis *in vitro*); and those skeletogenic mesenchyme mRNA sequences not represented in non-skeletogenic sea urchin embryo cells. A large set of relevant cDNAs are now being recovered. *(iii) Genomic approach:* Isolation of DNA fragments which share *cis*-regulatory elements already characterized functionally in the skeletogenic SM50 gene. We

found that the genomic DNA region that contains the *SM50* gene also includes a second gene which appears to encode another skeletogenic matrix protein. The two genes are linked at a distance of about 12 kb. Based on the molecular weight of the implied protein the gene is termed *SM37*. The *SM37* protein includes a long tandem sequence of short glycine-rich repeats that is similar to the glycine-rich repeats included in the *SM50* protein and several other skeletal matrix proteins, though the sequence of the *SM37* repeats is distinct. The overall structure of the *SM37* protein is similar to *SM50* as well. However, *SM37* is only about 30% identical in amino acid sequence to *SM50*, and coding region probes behave as a single copy sequence under standard conditions. The *SM37* gene includes the same *cis*-regulatory elements as the *SM50* gene, though in a different order with respect to transcription. This gene is regulated coordinately with *SM50* during development, and like *SM50* is expressed exclusively in skeletogenic mesenchyme lineages.

**CONCLUSIONS:** Molecular biological approaches are far superior to protein biochemistry and microsequencing for isolation of genes encoding proteins of the echinoderm skeletal biomineral. Of the molecular biology approaches, differential array screening is the most potent, and will probably yield all the skeletogenic genes used in the sea urchin embryo. By screening for elements of DNA that contain the known skeletogenic *cis*-regulatory sequences which control the *SM50* gene, we found a previously unknown gene which surprisingly is linked to *SM50* in the genome. This raises the possibility that there could be a more extensive set of skeletogenic genes linked in the genome, which could be isolated by direct genomic analysis.

**SIGNIFICANCE:** To bring analysis of the sea urchin biomineralization system to a new and more advanced level, the most important thing that needs to be done is to recover cDNA clones encoding the major proteins of the skeletal matrix. This would enable studies of the structure of matrix by mutation of the individual proteins; of the deposition of the matrix by overexpression or blockade of expression of specific genes; and it would make it possible to create matrices of different properties by combining specific sets of proteins produced by recombinant methods with mineral elements.

#### PUBLICATIONS:

- Lee, Y.-H., Britten, R. J. and Davidson, E. H. *SM37*, a new skeletogenic gene of the sea urchin embryo isolated by regulatory target site screening. *Dev., Growth & Differ.* **41**, in press.
- Lee, Y.-H., Huang, G. M., Cameron, R. A., Graham, G., Davidson, E. H., Hood, L. and Britten, R. J. EST analysis of gene expression in early cleavage-stage sea urchin embryos. *Development*, in press.